SCIENCE AND BUSINESS

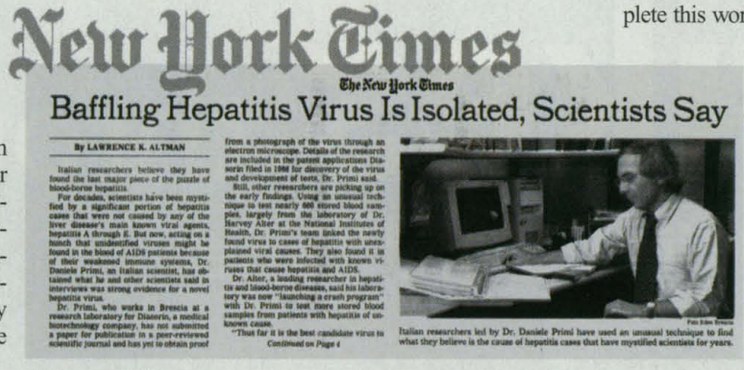
Report of New Hepatitis Virus Has Researchers Intrigued and Upset

American Standard, a company best known for making toilets and air conditioners, has taken a beating from stockholders and financial analysts for starting a money-losing biotechnology company, DiaSorin. Critics may have been somewhat mollified on 20 July, however, when *The New York Times* revealed that DiaSorin scientists had found a putative new hepatitis virus. The new agent could be responsible for many cases of the disease that for decades have baffled scientists—and it could form the basis of a lucrative test to screen blood supplies.

But the company's announcement, which came on the eve of disclosure of its second-quarter financial results, has attracted sharp criticism from hepatitis researchers. The findings have not been submitted for publication, nor have they been presented at scientific meetings. "It angers everybody," says the National Institutes of Health's Harvey Alter, who provided the company with blood samples that were key to linking the virus to hepatitis. "It has no scientific validity at all to publicize things before you're ready to publish. All the scientists involved tried to stop it."

If they pan out, the findings could have important public health implications. Researchers have linked five viruses, designated hepatitis A through E, to the liver inflammation known as hepatitis. But they have long suspected that other pathogens also cause the disease, because many people who develop hepatitis test negative for all the known hepatitis viruses. Blood-screening practices for other pathogens have already reduced new cases of transfusion-associated, unexplained hepatitis to near zero—presumably because likely carriers of the unknown pathogens have been screened out. But Alter says about 10% to 20% of past cases of acute hepatitis remain unexplained, as well as 30% of chronic hepatitis cases and 50% to 60% of cases that lead to rapid liver failure.

By fishing for new pathogens in the blood of injecting drug users who have AIDS, immunologist Daniele Primi and colleagues at DiaSorin's Biomedical Research Center in Brescia, Italy, may have identified the culprit. Alter is impressed with the results he has seen so far. "It has the feel of something that has potential causality," says Alter. But he quickly adds: "There's a lot more that needs to be done, though." For example, the company has yet to grow the virus in a laboratory culture, photograph it with an electron microscope, or show that it can infect chimpanzees and cause liver damage.



New York Times

The New York Times

Baffling Hepatitis Virus Is Isolated, Scientists Say

By LAWRENCE K. ALTMAN

Italian researchers believe they have found the last major piece of the puzzle of blood-borne hepatitis.

For decades, scientists have been mystified by a significant portion of hepatitis cases that were not caused by any of the liver disease's main known viral agents, hepatitis A through E. But now, acting on a hunch that unidentified viruses might be found in the blood of AIDS patients because of their weakened immune systems, Dr. Daniele Primi, an Italian scientist, has obtained what he and other scientists said in interviews was strong evidence for a novel hepatitis virus.

Dr. Primi, who works in Brescia at a research laboratory for Diasorin, a medical biotechnology company, has not submitted a paper for publication in a peer-reviewed scientific journal and has yet to obtain proof from a photograph of the virus through an electron microscope. Details of the research are included in the patent applications Diasorin filed in 1998 for discovery of the virus and development of tests, Dr. Primi said.

Still, other researchers are picking up on the early findings. Using an unusual technique to test nearly 600 stored blood samples, largely from the laboratory of Dr. Harvey Alter at the National Institutes of Health, Dr. Primi's team linked the newly found virus to cases of hepatitis with unexplained viral causes. They also found it in patients who were infected with known viruses that cause hepatitis and AIDS.

Dr. Alter, a leading researcher in hepatitis and blood-borne diseases, said his laboratory was now "launching a crash program" with Dr. Primi to test more stored blood samples from patients with hepatitis of unknown cause.

"Thus far it is the best candidate virus to

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Italian researchers led by Dr. Daniele Primi have used an unusual technique to find what they believe is the cause of hepatitis cases that have mystified scientists for years.

Primary publication. American Standard went to the media before submitting the findings to a journal or discussing them at a scientific meeting.

Primi himself says he urged the company not to announce his findings through the media. "I don't understand myself why it came out like this," says Primi, who adds that he has not submitted anything for publication because of patent concerns. The company, he says, filed a European patent application in November 1998, and corporate lawyers told him he had to wait 1 year before submitting a detailed scientific report about the virus and evidence of causality. "Unfortunately, science becomes a business," says Primi.

Company executives are defending their decision with unusual candor. They first mentioned the virus discovery in a cryptic press release issued in February, and they say they were duty bound to discuss it further when they disclosed their second-quarter earnings on 21 July. Company officials acknowledge that they approached *The New York Times* with the story shortly before that disclosure. "If we have a discovery that does have a significant impact on value, we have to share that with our stockholders," says Raymond Pipes, a vice president of investor relations at American Standard Companies, which grosses more than $6 billion a year. Pipes says the company has promised to decide by the end of the year what it will do with DiaSorin, which last year grossed $98 million and posted a $21 million loss. "Almost certainly that will involve not making it part of American Standard."

Jorge Leon, chief of the company's medical division, says the discovery led DiaSorin to request a budget increase, which American Standard had to explain to investors. "This is a very expensive enterprise," says Leon. "We still need to develop a larger effort to complete this work." As for why the company did not present the findings via the traditional scientific routes, Pipes says "we have the DNA sequence of the virus and, frankly, we don't want to reveal that data in the public domain until we have to."

Leading hepatitis researchers say they do not know what to make of the press reports. "I think this is a terrible way of doing this," says Leonard Seeff, a prominent hepatitis epidemiologist at the National Institute of Diabetes and Digestive and Kidney Diseases. "Things should be published in an appropriate forum so people can see the legitimacy of this." Robert Purcell, a hepatitis virologist at the National Institute of Allergy and Infectious Diseases, is equally circumspect. "Why should I believe them?" he asks. "You can be fooled by these things."

Although the company will not discuss many details, Leon allows that the virus contains DNA, not RNA, and shares less than 50% homology with any known virus. Primi says he looked for the virus in injecting drug users who had AIDS because he thought they would be infected with many pathogens and their HIV-impaired immune systems could not keep those infections at bay, resulting in high levels of any mystery agent in their blood. "To be honest with you, this was a project on the side," laughs

Primi. "It was the kind of thing you do where if it comes out with something, fine; if not, who cares."

To find the virus, Primi and his coworkers used random stretches of DNA called primers to fish DNA out of the blood samples of both the injecting drug users with AIDS and healthy controls. From a patient with the initials SEN, they found a large amount of an unknown virus, which they called SEN-V. Preliminary tests for the same agent in blood samples from patients with non–A-E hepatitis, provided by Mario Rizzetto of the University of Torino in Italy, suggested that they might have isolated the elusive hepatitis virus. Alter then sent Primi coded blood samples from both healthy people and those with non–A-E hepatitis. Primi found the virus in 10 of 12 people with transfusion-associated non–A-E hepatitis, four of 50 transfused people who did not develop disease, and one of 49 people who were not transfused. DiaSorin says the researchers have now analyzed nearly 600 blood samples, finding additional evidence for the virus in 13 of 19 people with unexplained chronic hepatitis.

If Primi and his colleagues have identified the non–A-E hepatitis virus, "it could potentially explain a lot of hepatitis," says Alter. But "the clinical relevance will be whether this virus can be shown to cause chronic liver disease," he points out. "Scientifically, I think it's sound. But it still can fall through. It was not a wise scientific decision to publicize this. It was an economic decision. I would have wanted a lot more data."

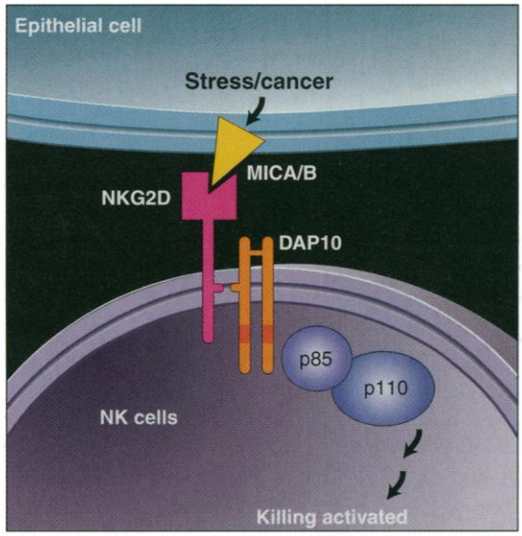
–JON COHEN

IMMUNOLOGY

A Trigger of Natural (and Other) Killers

When the immune system goes to war against invading pathogens or the insidious internal attack of cancer cells, it can deploy an arsenal of weapons. Some, like the antibody-producing B cells or the T type of killer cells, only attack when set off by a specific antigen. But others, such as the so-called natural killer (NK) cells, are far less picky; they eliminate a variety of infected or cancerous cells. How NK cells are triggered to mount such sweeping attacks, while remaining able to tell friend from foe, has long puzzled immunologists. Now, parts of that mystery appear to be solved.

On page 727, a team led by Thomas Spies at the Fred Hutchinson Cancer Research Center in Seattle reports that it has identified the molecular trigger that may help some NK cells pick their victims, as well as the receptor that recognizes that molecule, a protein called MICA. Other receptors appear to be involved in NK activation, but their molecular triggers are largely unknown. The identification of MICA as the activator of the new receptor is particularly interesting because it suggests that the receptor is key to NK cells' specificity. MICA appears to be switched on in cancer cells or in cells under stress, as may happen when they are infected by a virus. And in a second report, on page 730, a team headed by Joseph Phillips and Lewis Lanier of the DNAX Research Institute in Palo Alto, California, identifies the internal signaling pathway through which the MICA receptor tells NK cells to activate their killing machinery.



Making contact. The MICA/B proteins on infected or cancerous cells serve as destruction tags that can be recognized by NK cells. The DAP10 adapter protein then passes the killing signal to other proteins (p85 and p110) in a signaling pathway not used by other NK receptors.

To cellular immunologist Lorenzo Moretta of the University of Genova in Italy, it "makes perfect sense" that MICA is a trigger for NK cells. "In normal cells MICA is not expressed; it's only turned on when something goes wrong," he says. Together, the findings may someday help researchers design drugs that beef up NK responses to cancer or infections.

The road that led to the current discoveries actually began with another cell type—the oddball γδ T cells, which constitute less than 10% of all T cells. The antigen-binding receptors (TCRs) of the much more common αβ T cells operate on a dual-recognition system; they are triggered by antigens displayed on the surface of antigen-presenting cells in conjunction with major histocompatibility complex (MHC) proteins. The γδ TCRs don't require MHC proteins for their activation, however.

Indeed, researchers weren't sure what activates some γδ T cells, but about a year ago, Spies's team found that a population of γδ cells that live in the intestinal lining are triggered when their TCRs contact either of two MHC-related proteins with hitherto unknown functions: MICA and its close relative, MICB. These proteins, the Spies team showed later, seem to be switched on in many tumors of the lung, breast, and other organs—implying that some of the cell-killing γδ T cells act as tumor watchdogs by spotting MICA/B-bearing cancer cells through their antigen receptors.

Because NK cells are themselves well known as tumor-cell killers, Spies and his colleagues, Stefan Bauer and Veronika Groh, wondered whether these or other immune cells would also be capable of recognizing MICA. To their surprise, the researchers found that MICA binds to almost all NK cells. Evidence that MICA marks the cells that display it for killing came when the researchers engineered cells that normally resist killing by NK cells—and that don't make MICA—to display the protein on their surface. NK cells, they found, made short work of these newfound targets.

Bauer also bagged the gene for the MICA receptor, by "subtracting" the active genes in cells that didn't bind MICA from the active genes in cells that do. Out of five candidates, "only one made sense," says Spies. This was the gene encoding a known NK cell protein called NKG2D, whose structure indicated that it is a surface receptor. Further work confirmed that NKG2D is indeed the MICA receptor. For example, Groh found that MICA-positive cancer cells could be protected from NK cells by antibodies against either NKG2D

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